STUDIES ON A VIRUS CAUSING MOSAIC MOTTLING DISEASE ON CAPE-GOOSEBERRY (*PHYSALIS PERUVIANA* L.)

DISSERTATION

submitted in partial fulfilment of the requirements
for the award of the degree of

Master of Philosophy

IN

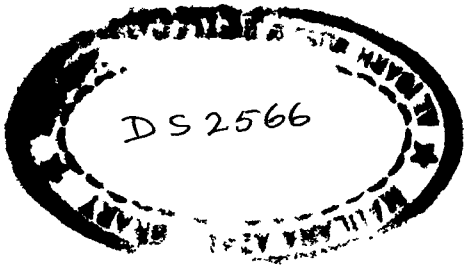
AGRICULTURE

(PLANT PATHOLOGY)

BY

DOOMAR SINGH

INSTITUTE OF AGRICULTURE
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
1994



QAMAR A. NAQVI
M.Sc., Ph.D., F.P.S.I.
CIES, Paris, France.

DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH - 202002
(INDIA)

Dated : 21.2.95

C E R T I F I C A T E

Thi is to certify that the dissertation entitled **"STUDIES ON A VIRUS CAUSING MOSAIC MOTTLING DISEASE ON CAPE-GOOSEBERRY (PHYSALIS PERUVIANA L.)"** submitted by **Mr. DOOMAR SINGH** to the Institute of Agriculture, Aligarh Muslim University, Aligarh in partial fulfilment of the requirements for the degree of **MASTER OF PHILOSOPHY IN AGRICULTURE** (PLANT PATHOLOGY), is a faithful record of the work carried out by him under my supervision and guidance.

[QAMAR A. NAQVI]

ACKNOWLEDGEMENT

I wish to take this opportunity to express my deep sense of gratitude and indebtedness to my respected teacher and supervisor **Dr. Qamar A. Naqvi**, Department of Botany, A.M.U., Aligarh, for suggesting me a problem of vital interest and for his skilful guidance, fruitful discussions and sympathetic behaviour throughout the course of this work.

I alos grateful to **Prof. M. Shamim Jairajpuri**, Director, Institute of Agriculture and Department of Botany, A.M.U., Aligarh for their assistance and cooperation which enabled me to complete this task.

It is a distinct pleasure for me to acknowledge and record heart felt gratitude to all my friends and well wishers for the costant inspiration and moral support.

I would miss my duty if I do not make a mention of my **Parents** whose sacrifices and blessings gave me impetus to achieve this goal.

[DOOMAR SINGH]

CONTENTS

Contd.....2.

****

CHAPTER - 1

INTRODUCTION

I N T R O D U C T I O N

Health depends on the balanced diet as a country depends on the healthy citizens. The balanced diet includes carbohydrates, proteins, minerals, salts, starch, water and vitamins. These substances are present in the vegetables, pulses, eggs, meat, cereals, milk, vegetable oils, milk products and in fruits. Cabohydrates, minerals, salts etc. are obtained from vegetables, proteins by eggs, pulses and meat, and starch by cereals but most of the vitamins are present in the fruits and are necessary for immunization of the animals. The vegetalbes, & vegetable oils, pulses, cereals, milk and milk products are main parts of the vegetarion diet but the fruits are necessary for both vegetarions as well as non - vegetarions, and therefore, the importance of fruits automatically increases for healthy human beings. A **proverb is absolutely true that - "One apple one day, doctor keeps away".**

The cape - gooseberry (Physalis peruviana L.) has sour-sweet taste and known by different names in different Indian languages such as in Hindi it is called- Tipari, in Beng - Tipariya, Mar. - Phopti; Guj. - Motipopti, Tel.- Buddabasara; Tam.- Tholthakkali; Kan. Gudde hannu; Punjab & Delhi - Rasbhary, Mewar reshberry,

Maharashtra - Chirput, Chirboti, tankari. Its native place is tropical America and later introduced in India and other countries and grown both in the plains and hills.

The plant is widely grown in India for its edible fruits; the chief source of commercial supply is reported to be Uttar Pradesh, Rajasthan, and Punjab, other localities reported are, Poona, coonoor and Araku Valley (A.P.). It is frequently found wild as an escape from cultivation. The plant favours a warm weather preferabley with good rains during the growth period, but a comparatively drier weather is essential at the time of maturity of fruits. It is grown in varying types of soil ranging from sandy loam to laterite. Planting is best done in September - October.

The plants can be grown pure or as an intercrop in orchards. The crop matures in about three months after transplantation. In south India two pickings can easily be obtained in a year where as in north India, the fruits are available from January to April. Fallen berries are said to be more uniform in maturity than handpicked ones. The yield of fruits per plant is said to be about 2-4 Kg, the yeild per hectare as a intercrop being 1,650-2,225 Kg; as a pure crop the yield may go up to a maximum of

33,600 Kg, per hectare. As an intercrop the duration of the crop is one year, while as a pure crop it can be extended upto 3-4 years, after which it becomes uneconomical.

The fruits are pleasantly acidic with a good flavour and are relished as a table fruit. Analysis of *edible portion (87%) of the fruits gave; moisture* 82.9; Protein, 1.8; fat; 0.2 Carbohydrates, 11.1; fibre, 3.2, and mineral matter 0.8%; Calcium, 10; magnesium. 31; Phosphorus, 67; Phytin phosphorus, 18; iron, 2.0; ionisable iron, 0.9; Potassium, 320; Copper 0.19 and sulphur, 43 *mg/100g; acid base balance (ml of N/10 alkali), 39.* The fruits are a good source of carotene and ascorbic acid. They contain carotene (as vitamin A), 2,380 I.U.; thiamine, 0.05; riboflavin, 0.02; nicotinic acid, 0.3; and ascorbic acid, 49 mg/ 100g. Ascorbic acid content varies with maturity of the fruits; unripe fruits 48.6-53.8 mg/100g. of ascorbic acid. The presence of a bioflavonoid is reported in the fresh fruit (Khan and Gowder, 1959, Gunju & Puri, 1959).

A fairly large quantity of gooseberries is reported to be used for making jam in India. The juice from the ripe fruits contains considerable quantity of pectin. The filtered juice gave the following chemical

composition: total solids, 14.4; total ash (mainly potassium and sodium carbonates), 0.97: total acidity (as anhydrous citric acid), 2.2; free glucose, 4.4; and total invert sugars after hydrolysis, 16.97%. The chief acid is citric acid, But malic and tartaric acids are also present (Lal, 1936).

The husk of the fruits has a bitter taste. It contains a mixture of potassium Chloride and potassium citrate a phytosterol, a bitter amorphous glucoside, trace of a Pungent alkoloid, tannins and pholobaphenes (Lal,1938). Leaf contains chlorogenic acid. Leaf infusion is used in abdominal disorders. The heated leaf is applied as a poultice.

The cape-gooseberry is said to be subject to very few diseases. A mosaic disease is caused by a strain of the tomato mosaic virus has been reported from commercial plantations near Poona. Affected leaves and fruits are shed. The disease is spread by contact (Khan and Gowder : 1965).

A mosaic disease of cape - gooseberry, Physalis peruviana L. observed on the basis of host range, physical properties, symptamatology, transmission by insects and serology, indicated that the virus belonged to the Cucumber mosaic virus group (Singh, 1968; Nariani &

Sharma, 1971; Singh *et al*, 1975). Several other viruses have isolated from the *Physalis peruviana* e.g. tobacco mosaic virus (Chamberlain, 1937) tobacco etch virus (Holmes, 1944; Costa, 1944, Mcclean, 1954), alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic, potato X, tobacco mosaic and tobacco ringspot (Horvath, 1974) were found to cause locally on *P. peruviana*. This plant was also reported as a symptomless carrier by the following authors e.g. PLRV by Natti *et al*, 1953; tobacco stem mottle virus, schmelzer, 1955; and PSTV by Muriel *et al*, 1964.

****

CHAPTER - 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cape-gooseberries (Physalis peruviana L.) are attacked by a numberof virus belonging to different plant virus groups. The most common symptoms displayed by the virus infecting cape-gooseberry plants are mosaic mottling leaf curling and an overall reduction in the foliage.

The mosaic disease of cape-gooseberry was first reported from India by Singh (1968) from Gorakhpur; Naraini and Sharma (1971), New Delhi; and by Singh et al (1975) from Bangalore. The mosaic mottling disease of cape-gooseberry has not been reported from other countries.

Cape-gooseberry is a host of a large number of virus such as TMV (Tobacco mosaic virus: Costa, 1944; Beake, 1965; Chamberlain, 1968 and Harvath, 1975), CMV (cucumber mosaic virus: Doolittle, 1926; Walker, 1926, and Johnson, 1926), ToMV (Tomato mosaic virus : Gardner, 1828; Bold 1932; Sakimura, 1954; Graca et al 1986), PLRV (Potato leaf roll virus: Natti et al 1953), Potato virus X ardy (Sakimura, 1958; Harvath, 1970; 1974; Singh, 1978), TSMV (tobacco Stem mottle virus: Schmelzer, 1955), tobacco etch virus (Holmes, 1942; Mc Clean, 1954; 1956; Costa, 1944; Anderson, 1954), TRSV (tobacco ring spot virus :

Harvath, 1974), Bunchy top disease of tomato (McClean, 1931, 1932, 1935) etc. These viruses have been identified by their host range, symptoms, biophysical properties and electron microscopy.

Tobacco mosaic virus was identified in the Hungary by Costa (1944), Harvath (1975); in New Zealand by Chamberlain (1968) and by Beake (1965). Tomato mosaic virus on cape-gooseberry was identified in India by Gardner (1928). Tomato spotted wilt virus was first identified in Australia by Bald (1932), in Hawaii by Sakimura (1954) and in South Africa by Graca et al (1986). Bunchy top virus of tomato was also infected cape-gooseberry in South Africa (Mc Clean, 1932, 1935).

Cucumber mosaic virus (CMV) is a very common virus found on cape-gooseberry. It has been reported from U.S.A. by Doolittle (1926), by Walker (1926) and by Johnson in the same year. Serological relationships indicated that the virus belonged to the cucumber mosaic virus group (Mariani & Sharma, 1971), Singh 1969). But Singh et al (1975) reported that it did not react positively with the antisera of cucumber mosaic virus.

Further the overwintering and dissemination of cucurbit mosaic virus has been studied by Doolittle and

Walker (1926). They reported that the cucumber mosaic also overwinters in the roots of catnip (Nepeta cataria) and certain perennial species of Physalis.

Johnson (1926) reported mosaic diseases on differential hosts. With four additional mosaics namely (1) cucumber mosaic (2) Petunia mosaic (3) Speckled tobacco mosaic and (4) mild tobacco mosaic. The symptoms of these mosaics are quite distinct on tobacco seedlings, except in the case of petunia and speckled tobacco mosaic, which appear to produce an almost identical conditions. The virus of these two forms of mosaic can be differentiated on Nicotiana glutinosa, petunia, pokeweed (Phytolacca decandra), and probably on Physalis, as well as by their comparative longevity outside the host. On the basis of symptoms developed on Physalis , it may be regarded as a good differential host for this type of mosaic.

Gardner (1925) reported that only the typical tomato mosaic was carried in perennial weeds such as Physalis, the other virus being frequently present in healthy as well as in mosaic potato plants. A study of the effects of filiform or fern-leaf type of mosaic on the floral anatomy snould important modifications of the calyx and corolla lobes in additions to the extreme reduction of the leaves.

Deighton (1929) studied a leaf Crinkle, possibly belonging to the virus group of diseases, affected cape-gooseberry (Physalis peruviana), showed symptoms such as chillies.

Spotted wilt of tomatoes was investigated by Bald (1931). He observed that some of the leaves are mottled and deformed by this virus. Mottled fruits some times occured on plants showing no other definite symptoms of the disease, while diseased plants sometimes found bearing fruits without mottling. The virus has longevity in Vitro of six hours at room temperature, and TIP 42°C. The most important evidence of his experiment was that the adult Frankliniella insularis thrips were able to transmit the disease. Under medium temp. conditions the incubation period of the virus in the larva was thought to be probably between five and seven days. Spotted wilt virus was experimently transferred from tomatoes to tobacco and 14 other species of Nicotiana, as well as to seven species of Solanum, capsicum annuum, Datura stramonium, Hyoscyamus niger, Lycium, ferocissimum, Petunia hybrida, Physalis peruviana, Salpiglossis sp., Schezanthus sp., Aster sp. and Chrysanthimum sp.

McClean (1931a & 1931 b) studied the Bunchy top disease of tomato and found that the symptoms were macroscopically closed to the American curly top of tomato

described by Chupp (mannual of vegetable garden diseases; p.597). The most characteristic symptoms are severe stunting, stem necrosis, downward curling and abnormal unevenness of the surface. A peculiar feature of the disease was that the check caused by it to the elongation of the internodes is limited to the early stages of infection. Diseased plants produce normal flowers and normal setting of the fruits, but the yield is considerably reduced and the tomatoes that reach maturity are small and frequently destroyed, these fruits are either seedless or contain a few small seeds. The disease was successfully transferred to Physalis peruviana and back to tomato. The main source of the infection is the presence of diseased tomato plants of previous crops and Physalis. The tomato bunchy top virus is beleived to be similar to that of tobacco mosaic. Cape-gooseberries (Physalis peruviana) are very susceptible. The disease can be controlled by the use of an insecticide, such as lime-sulphur and lead arsenate in the seed-bed (McClean, 1931b).

The serum reactions as an aid in the study of filterable viruses of plants was given by Beale (1934). Extracts taken by the author from Solanum melongena S. sisymbrifolium, Physalis peruviana, capsicum minimum and C. frutescense [Capsicum annuum] affected with Johnson's

tobacco mosaic virus No. 1, and the Turkish tobacco affected with aucuba mosaic and attenuated tobacco mosaic, gave a possitive precipitin reaction with antiserum to tobacco virus No. 1 , while extracts from other Solanaceous plants affected with mosaic diseases other than the tobacco mosaic reacted negatively with the antiserum. A quantitative relation was found to exist between the antigenic content and the active virus concentration of the extracts used in the tests.

Mc Clean (1935) studied the host range of bunchy top disease of tomato. He showed that this disease transmitted to Solanum aculeatissimum, S. nigrum, S. sodomacum, Nicandra physaloides, Physalis angulata, P. viscosa, tobacco, eggplant, cape-gooseberry (P. peruviana) petunia, pepper [Capsicum annuum], and potato.

Chamberlian (1937) studied the appearance, cause and control of tobacco mosaic virus. Experiments showed that infection when occuring shortly after the plants had been set out in the field caused 44 and 78% reduction of yield in a virginian and a Burley variety respectively. Infection later in the season caused corrosponding losses of 24 and 25%. Other hosts attacked locally are tomato, black-night shade (Solanum nigrum), cape-gooseberry (Physalis peruviana), Turkestan tobacco, eggplant, and chilli (capsicum annuum) of which only the first two are likely to play any part in transmission to tobacco. The

virus was transmitted by men who previously performed the operations on mosaic plants then the same operation performed on healthy plants, developed 83% infection after three weeks. The author showed 11% infection carried out from the infested soil in previous season.

The association of leaf curl disease of tobacco with white fly (Trialeurodes natalansis) was studied by Mc Clean (1940). He reported that the disease was transmitted to tobacco, Datura stramonium, tomato, current tomato (lycopersicum pimpinellifolium), N. glutinosa, Nicandra physaloides and possibly also to Physalis peruviana, Helichrysum monstrosum and Zinnia elegans. Tobacco and various solanaceous hosts were also infected by graft transmission but not by mechanical inoculation or through the seeds. During the transmission of the virus from plants with severe leaf curl, apparently weaker strains of virus arose, which produced milder symptoms on tobacco and other hosts.

Quantitative measurement of a strain tobacco-etch virus was studied by Holmes (1942). He showed that under appropriate environemtnal conditions well marked necrotic primary lesions developed on Physalis peruviana leaves five to ten days after inoculation with the severe etch strain of tobacco etch virus which induced on tobacco both

chlorotic mottling and inticrate pattens of fine white lines simulating etching, this feature being absent from the other hosts of the virus. The number of lesions produced by severe etch virus were used for quantitative measurements and it was found that Nicotiana glutinosa the best source of inoculum. Infectivity of this virus retained in acid-buffer solutions (pH 4.5 to 6.0) at temperature just above and below freezing. The TIP was 53°c at 10 min. exposing or 51°C, for half an hour exposing. The Physalis peruviana was also used for the quantitative studies with carborundum and its use in local lesion test for tobacco etch virus (Casta, 1944).

Sakimura (1953) studied a mixed natural infection of potato virus y and (tomato) spotted with virus in the field planting of tomato in Hawaii in 1950 and 1951. The TIP was between 53° and 66°C for a 10 minutes exposure; longevity in Vitro ranged from 48 to 72 hrs at 25°C to 29°C and from 66 to 82 days at 0° to 3°C and the DIP was between 1 in 300 and 1 in 1000. The virus was transmitted by sap inoculation to eggplant, Solanum nodiflorum, Nicotiana glutinosa, N. rustica, tobacco, Physalis peruviana, chilli (capsicum frutescens) Petunia, Nicandra physatoides and tomato; Nicotiana rustica was readily affected by Myzus persicae after a one-to-two days acquisition and a three - to - four days infection feeding of 5 to 15 apterous young aphids per plant.

Natti et al (1953) found that Physalis peruviana was a symptomless host of potato leaf roll virus. It was transmitted by means of Myzus persicae. Attampted recovery of the virus was made by feeding aphids for five to six days on the test plants and then transferring them to indicator plants. Several solanaceous species were symptomless; Atropa belladona , eggplant, Lycopersicon pimpinellifolium, Nicotiana glutinosa N. rustica, Physalis peruviana, Solanum citrullifolium, S. intigrifolium and S. nigrum.

Mc Keen (1954) observed that the percentage of successful transmissions of tobacco etch virus obtained by rubbing the leaves of Physalis peruviana with sap from infected chilli was small compared with transmissions from tobacco, and the addition of chilli extract reduced the number of primary lesions on Burley tobacco to one-seventh or one eighth of the numbers given by viruliferous tobacco sap alone.

A leguminous host Cassia tora as well as Physalis peruviana could be used in immunological studies of tobacco etch virus (Anderson; 1954) for former being of particular value in resisting systemic infection by several other viruses to which P. peruviana is susceptible. The development of systemic necrotic

patterns, which seriously complicated the making of accurate lesion counts, may, possibly, be avoided if tests are conducted under cooler conditions.

Nariani (1954) reported that Physalis peruviana L. as a host of tobacco leaf curl virus, causing stunting of the plants, and curling of the leaves with production of vein enations on the lower surface.

Schmelzer (1955) represented 41 genera of 21 families and included spinach, aster, Zinnia elegans mustard, broad beans, peas, flax, buck wheat, Reseda odorala, Antirrhinum majus, Cyphomandra belacea, Nicandra physaloides , Petunia hybrida, Physalis peruviana and Pansy infected by tobacco stem mottle virus. Author observed that P. peruviana remained completely symptomless, though systemic infection was demostrated by repeated tests.

Mc Keen (1956) reported tobacco etch virus on tobacco and Physalis peruviana and showed the inhibitory effect of the expressed sap of chilli, pepper leaves on virus infection. The inhibitive components of chilli sap was thermolabile, resistant to ageing and drying in Vitro, and does not pass through a cellophane membrane, these properties suggesting a protein. In chilli sap that precipitated by 95% ethanol or ammonium sulphate was less inhibitory than raw sap.

Muriel et al (1964) observed that Petunia hybrida var. Flaming Velvet, Nicotiana rustica, N. debneyi and Physalis peruviana were symptomless host of the potato spindle tuber virus(PSTV).Tomato bunchy top virus and PSTV have the similar biophysical properties. Symptoms of both viruses (tomato bunchy top & PSTV) both transmitted through the true seeds of P. peruviana were thought to be the same virus or Str. of the same viruses confirmed by serologically and by the electron microscope (Benson et al, 1965).

Singh (1968) investigated a mosaic disease of cape- gooseberry (Physalis peruviana L.) and found that the disease was similar to that caused by cucumber mosaic virus. The mosaic mottling, distortion, malformation and reduction of lemina were the main symptoms of this disease and the virus was sap transmissible and readily by Aphis gossypii and Myzus persicae.

Horvath (1970) reported that the cape gooseberry was a symptomless carrier of potato virus X and 4 Str. of Y. A mosaic disease of cape- gooseberry (Physalis peruviana) on the basis of host range, physical properties, transmission by insects and serological relationships indicated that the virus belonged to the cucumber mosaic virus group (Nariani and Sharma; 1971).

Horvath (1974) studied the reaction of Physalis species to plant viruses. Physalis peruviana var. macrocarpa found locally and symstemically susceptible to alfalfa mosaic, cucumber mosaic, potato aucuba mosaic, potato x, tobacco mosaic and tobacco ringspot viruses while that plant was immune for bean common mosaic, potato M, potato S, radish mosaic, tobacco necrosis and turnip yellow mosaic viruses. P. floridana Rydb. and P. peruviana L. were immune to bean common mosaic, potato M and S, radish mosaic, and turnip yellow mosaic viruses (Horvath; 1974b) and the local susceptibility to tobacco rattle virus was noted.

Singh et al (1975) identified a virus as new which caused a mosaic disease of cape- gooseberry. The symptoms on Physalis peruviana were as like as observed by Singh (1968) and also reduced fruits and flowers. Only Myzus persicae transmitted the virus. They showed that the virus could not react positively with the antisera of cucumber mosaic, tobacco mosaic, tobacco ringspot, tobacco etch virus, potato Y and potato X viruses.

The casual agent of the disease which deformed and yellowed the leaves and stunted the plants of tomato and Physalis was thought to be a virus or mycoplasma like organism transmitted by Trialeurodes vaporariorum (Herandez Roque et al; 1974).

Singh (1978) reported that Physalis floridana and 2 other Physalis species could be used to differentiate the closely related potato viruses A and Y.

Host plant reactions, some properties and serology of Peru tomato virus were studied by Fribourg (1979) and found that the weeds Nicandra physaloides, Physalis peruviana and Solanum nigrum were natural hosts of this virus. The DEP was 10^{-5}, TIP was 50°C after exposing for 10 min. in a water bath and longevity in Vitro was 4 days. Electron microscopy of infective sap showed long flexous particles c.775 nm. in length and typical of the poty virus group.

Graca et al (1985) reported tomato spotted wilt virus in commercial cape-gooseberry (Physalis peruviana) in S. Africa.

****

CHAPTER - 3

PLAN OF WORK AND METHODS

PLAN OF WORK AND METHODS

1. Maintenance of Virus inoculum :

1.1 Raising of Test Plants :

All the plants will be grown in clay pots of 4" and 6" diameter, filled with a mixture of soil, sand and compost in a ratio of 2:1:1. The soil mixture will be sterilized by autoclaving for one hour at a pressure of 20 lbs per square inch. The clay pots will be sterilized by rinsing in 4 per cent formalin solution and prepared by filling with sterilized soil mixture autoclaved 24 hrs. earlier and seived before use.

Cape-gooseberry (Physalis peruviana L.) plants and other plants belonging to family solanaceae will be sown in 12" clay pots for raising seedling. Other plants belonging to different families having non-transplantable will be raised singly by direct sowing in clay pots. However, formalin rinsed wooden trays (18" x 18" x 5") containing sterilized soil mixture will be used in raising seedlings of various plants. Young seedlings of uniform size will be transplanted singly to 6" and 4" pots containing the autoclaved soil, sand and compost mixture.

For inoculations the plants will be used two weeks after transplantation. All the plants will be raised and kept in an insect proof glass house (room temp. and normal day length) and given a uniform care such as fertilizer, water, insecticide and other requirements.

1.2 Virus Culture :

The virus culture will be obtained from naturally infected plants showing symptoms of virus infection and maintained on suitable propagation host by mechanical (sap) inoculations. Attempts of single lesion inoculations will be made to maintain a pure virus culture. Virus/es not transmitted mechanically will be knowingly omitted and only mechanically transmitted virus/es will be taken for investigations. Once the culture of the virus/es has been maintained on suitable propagation host, it will be kept in active state by fresh inoculations at regular intervals on young propagation hosts.

1.3 Source of Inoculum:

Young leaves from infected propagation host plants will be used as source of inoculum. Inoculum will be prepared by macerating them in a mortar with pestle in 0.1M phosphate buffer pH 7.0. For each gram of leaf

material 1 ml of buffer will be used and the macerate will be filtered through two layered cheese cloth. The sap thus obtained will be used as standard inoculum.

2. Transmission :

2.1 Mechanical :

The fully expanded leaves of the plants to be inoculated will be dusted uniformly with carborundum 500 mesh as an abrasive and the standard inoculum will be applied gently but firmly on the upper surface of leaves with the help of forefinger by keeping the other hand beneath the leaf to be inoculated. The inoculated leaves will be rinsed with gentle stream of water before the inoculum on the surface of the leaves dries up. If the rate of transmission is not promising, some chemicals will be mixed with the inoculum so as to enhance the rate of transmission. Additive in the inoculum will include sodium sulfite, 2- mercapto - ethanol, ethylene diamine - tetracitic acid, sodium dielthyl- dithio - carbamate and thioglycolic acid either alone or in various possible combinations if needed.

2.2 Biological :

Attempts will be made to find out the vector/s of virus in the field, transmission by insects, soil, dodder

(cuscuta) spp.), seeds, grafts, nematode and pollen will be studied.

2.2.1 Insect Transmission:

2.2.1 (a) Transmission by aphids :

Adult aphids found transmitting the disease during preliminary investigations will be used to study aphid-virus relationship (non persistant, semi-persistant or persistant manner)

2.2.1 (b) Raising of virus free aphids :

Viviparous adults will be starved for 2, 4, 6 and 8 hrs at room temperature in a petridish and then placed upon a detached leaf or an appropriate healthy host in a petridish. The atmosphere inside the petridish will be made humid by covering the inner surface of the pertridish with wet filter paper. Newly born nymphs will be transferred to a fresh and healthy plant. The aphid colonies thus developed will be used as healthy colonies of the virus free aphids. The aphids from one plant to other will be transferred with the help of moistened tip of camel's hair brush type A, No.1. Colonies of virus free aphids will be raised on suitable host plant in cages having wooden frams. The top and the two sides of the cage will be closed by glass and the remaining sides will be

closed by wire gauze. A flourescent tube will be fixed in the cage to provide artificial light to the plants and to keep the aphids under long day conditions to get the apterous (wingless) aphids. The plants will be kept on a zinc tray and the bottom of the tray will be covered with a layer of moist sand to prevent the passing of the aphids through chinks between the tray and the rim of the cage.

2.2.1 (c) **Mode of Transmission** :

To establish the mode of transmission following procedure will be adopted.

(i) **Non-persistant** : Virus free aphids will be first starved for 4-8 hrs in a glass vial before an acquisition access feeding of 1-2 min on the detached leaf of the diseased plant placed on moist filter paper in a petridish. After acquisition feeding, 10-20 aphids will be transferred to each healthy seedlings of test plants for an inoculation feeding period of 24 hrs. The plants will be covered with lantern chimney having its top covered with muslin cloth to avoid aphids from escaping. The aphids, after the end of the inoculation feeding will be killed by spraying

an insecticide. The test plants will be kept in insect-proof glass house to observe the development of symptoms.

SUMMARY

1. Pre-acquisition starvation period ....4-8 hrs.
2. Acquisition access period -- 1-2 min
3. Inoculation access period ..... 24 hrs
4. Number of aphids / plant .... 10-20.

(ii) **Persistant** :- The virus-free aphids, without subjecting them to starvation will be allowed 24 hrs acquisition feeding time on diseased leaves placed on a moist filter paper in a petridish. After the completion of acquisition feeding, 10-20 aphids will be transferred to each test plant where they will be given an inoculation feeding period, aphids will be killed by spraying an insecticide. The test plants will be kept in an insect proof glass house to observe the development of symptoms. Back inoculations from the plants on which aphids were given inoculation feedings will be made on an appropriate diagnostic host.

SUMMARY

1.	Acquisition access period	24 hrs.
2.	Inoculation access period	48 hrs.
3.	number of aphids per plant	10-20.

2.2.2 **Transmission by white flies** :

(a) **Source of virus free white flies** : White flies (Bemisia tabaci Genn.) collected from (Clitoria turnatea will be caged on a healthy plant of C. turnatea for egg laying. After 10 days the adults will be removed from the cage. New white fly adults developing after 7-8 days would be further multiplied. Insect colonies so raised will be virus free and would be further multiplied and would be used for transmission studies.

(b) **Handling of White flies** : Same mehtod as used by Srivastva et al (1977) will be carried out for handling of white flies.

(c) **Transmission** : Non-viruliferous white flies would be allowed for acquisition and inoculation access period of 24 hrs. each on diseased and healthy plants, respectively. Cypermethrine (0.02%) will be sprayed to kill the white flies after inoculation. The test plants will be kept for a month for observation of symptoms.

2.2.3 Graft :

Attempts will be made for side wedge grafting. Infected scions will be grafted on healthy stock and kept under appropriate light and humidity conditions to allow successful union whichis necessary for transmission.

2.2.4 Dodder :

Seeds of dodder (Cuscuta spp.) will be germinated on moist filter paper placed in pertiplates and then transferred in 2" clay pots, sterilized with formalin (4%) and containing sterilized soil mixture when the plants are about 6" long, they will be trained on a suitable host plant susceptible to the virus being studied and the host plant (on which the dodder is being trained) will be inouclated after one week. When the dodder has been established on inoculated plant, a healthy test plant in another pot will be placed near the pot (having inoculated plant with dodder established on it) and the tips of the branches of dodder will be detached, placed in the axil of the healthy test plant and allowed to established there. The plants thus inoculated will be observed for the development of symptoms, if any for about 6 weeks. Back inoculation will be made on local lesion host to confirm the presence of virus (transmitted by dodder).

2.2.5 Soil Transmission :

Soil around the naturally infected plants will be collected from the field and sieved to remove roots and debris etc. Such soil will be divided into two parts. One part will be filled in a gumy bag and will automaticlaved at 15 lbs/inch^2 for 30 minute and the other part of soil will be lef unautoclaved and will be filled in pots as such. Seeds will be sown in pots containing sterilized and unsterilized soil. Plants of both the sets will be kept for observation in an insect proof glass house for a period of two months. Back inoculation tests will be carried out to ascertain the presence of viurs in them.

Any appereance of the symptoms in the seedlings grown in unautoclaved soil wil indicate the presence of soil borne vectors or the seed transmissible nature of virus. Soil borne vectors may be either, nematodes or fungi.

2.2.5 (a) Search of fungal Vectors :

Roots of naturally infected plants not watered since 2-3 days will be immersed in double distilled water for 15-30 minutes. The suspension will be tested in two ways.

1. Suspension (roots + water) will be poured around the roots of healthy test seedlings. Development

of symptoms on such test plants will indicate that the fungus carried the virus internally.

II. Fungal suspension obtained as described above will be mixed with the standard virus inoculum. The mixture will be allowed to incubate for 15-20 minutes and thereafter poured around the roots of healthy test seedlings. Symptom development if any will indicate the association of fungal vector carrying the virus externally on Zoospores.

2.2.5 (b) **Search for nematode Vector** :

Nematodes from soil samples usually Longidorus, Xiphinema and Trichodorus known as the vector of some of plant viruses will be isolated from the soil samples using cobb's method (cobb, 1918).

To assure whether the nematode isolated from the soil smaples carry virus or not, the following tests will be performed.

I. A drop of concentrated nematode suspension on a glass slide will be macerated with a glass spatula and inoculated on the leaves of local lesion host.

II. Nematode suspension will be poured around the roots of test plants. The plants will be kept for the observation of the symptoms for about one to two months.

Similarly varying number of nematodes isolated will be poured around the roots of virus infected plants in a insect proof glass house inoculated priorly. After an acquisition feeding of 15 days, infected plants will be uprooted and the healthy ones will be planted in the same pots. Plants will be kept for observation of symptoms. Back inoculations from all nematode inoculated plants will be made on a local lesion host.

In case of positive observations the studies will extended to identity the specific nematode species acting as vectors and its relationship with the virus under investigation.

2.2.6 Seed transmission :

A few inoculated plants will be kept till flowering and fruiting. After the seed maturation they will be collected and dried. A considerable number of seeds will be sown in the wooden trays containing sterilized soil mixture. The germinating seeds will be

counted. The number of healthy and diseased plant, if any, in the trays will be counted. To compare their percentage germination, seeds from the healthy plants will also be treated in the same way.

Seeds transmissible nature of the virus under study will be tested by the following method:

(a) By macerating the seeds from diseased plants in 0.1 M phosphate buffer pH 7.0, giving macerate a low speed centrifugation and inoculating the sap thus obtained on the local lesion host.

(b) By keeping the plants, developed from the seeds collected from diseased plants under insect proof glass house for about one month to observe the development of symptoms.

(c) By inoculating sap obtained from young seedlings developed from seeds collected from diseased plants on local lesion host.

3. <u>Host range Studies</u> :

Several species of plants, belonging to different families will be screened for the susceptibility to the cucumber mosaic virus. Standard inoculum will be used for inoculation of all plants. At a time at least five

plants of each species will be inoculated and the same number will be kept as a control. Plants at 5-6 leaf stage (cucurbits at seedlings stage) will be used and all the fully expanded leaves will be inoculated. The inoculated plants will be observed upto two months for the development of symptoms. The time, sequence and severity of the symptoms will be noted. Inoculated plants exhibiting no symptoms will be kept for about 8 weeks for observation. Back inoculations will be made to a test plants from all the inoculated plants.

4. Virus vector relationship:

In order to determine the relationship between the virus and the vector, the method would depend on the type of the vector group involved in transmission. However, in general, variabilities including number of insect per plant, different preacquisition starvation periods, varying acquisition and inoculation access periods will be worked out along with effect of moulting of insect on various retention and latent periods in the vector.

5. Effect of different buffers on the infectivity :

Various buffers (phosphate, borate, citrate, acitate, glycine. NaOH and tris- HCl) at different pH and molarities will be used and tested to find out the

most suitable one in which virus infectivity is retained upto longer type.

Young infected leaves will be macerated in a mortar with pestle using a buffer (any of the above mentioned) as extraction medium. The sap obtained after filtrating it through double layered cheese cloth will be inoculated on the leaves of local lesion host following the usual method of inoculation. All buffers will be tested in the same way, and a buffer at a pH and molarity in which virus infectivity is higher will be selected and used regularly as an extraction medium for the virus being used.

6. **Virus Concentration in different parts of the host** :

To determine the virus concentration in different parts of the host plant, 10-15 days earlier inoculated plants will be uprooted carefully and washed. The plants will be blotter dried.Root, stem and leaf tissues will be cut separately into pieces. Equal amount of root, stem and leaf tissue will be macerated separately in mortar and pestle using a suitable buffer. Sap obtained from each sample will be inoculated separately on a local lesion host using usual methods of inoculation.

7. **Selection of Suitable Propagation host and an assay host** :

To find out a suitable propagation host several plants, susceptible to the virus will be inoculated and showing most prominent symptoms will be selected. A plant exhibiting following characters will be selected.

i. Rapid seed germination and fast growth.

ii. Short incubation period of the virus.

iii. Peak concentration of the virus within a short period after inoculation.

iv. Absence of virus inhibitors and

v. More yield of infected tissue with good virus concentration.

Assay of virus will be carried on a local lesion host. To search out a local lesions several commonly used plants will be tested.

However, in case of non-availability of a local lesion host, assay tests of the virus will be carried out on a systemic host.

8. **Biophysical properties** :

To determine the dilution end point, thermal inactivation point and longevity *in Vitro*, methods

described by Noordom (1973) will be employed.

8.1 Dilution end-point (DEP) :

Young leaves of propagation host inoculated 8-10 days earlier will be crushed in a mortal with pestle and the sap will be obtained by squeezing the marcerate through two layers of cheese cloth. Ten fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) will be made of the sap by adding of double distilled water or phosphate buffer 0.1M, pH 7.0. From each dilution three test plants will be inoculated manually using carborundum 500 mesh as an abrasive. The leaves of the plants will be washed by a gentle stream of water immediately after inoculation. Local lesion developed on the leaves of the test plants will be counted after 5-6 days. In this way the dilution at which virus loses its infectivity will be determined.

8.2 Thermal inactivation-point :

The sap will be obtained by macerating the young infected leaves of propagation host in a suitable buffer, inoculated 8-10 days earlier, in a mortal with pestle. The macerate will be filtered through two layers of cheese cloth. The sap thus obtained will be divided into 10 aliquots of 5 ml. each and kept in thin walled glass tubes.

The tubes will be held in a water bath in such a way that the sap level will be slightly below the level of water in the bath. The different aliquots will be heated at 40, 45, 50, 55, 60, 65, ... 95°C for 10 minutes and cooled under running tap water, immediately after the treatment. Each heated aliquot will be inoculated to three test plants, and local lesion developed on the leaves will be counted after 5-6 days. One aliquot which will not be heated, will also be inoculated to three test plants, to compare the local lesion produced with other aliquots.

8.3 Longivity in Vitro :

(a) **In Sap** : The infected leaves of the propagation host will be homoginised in a mortar with pestle, while using a suitable buffer and the homogenate will be filtered through two layers of cheese cloth and the sap thus obtained, will be kept at room temperature (20-25°C). After every 6 hrs interval, a small amount of the sap will be taken and inoculated on the leaves of local lesion host/systemic host (in absence of local lesion host). The process will be counted on the inoculated leaves for each interval and the time after which the virus loses its infectivity will be recorded.

(b) **In dried leaves** :- The young infected leaves of the propagation host will be cut, in to small pieces and dried

over anhydrous calcium chloride in a desiccator. After 24 hrs interval, such pieces will be homogenised, by using a suitable buffer, in a mortal with pestle. The sap thus obtained after passing through two layered cheese cloth will be inoculated onto the leaves of local lesion host. This will be continued upto the time, until the virus loses its infectivity in the tissue dried over anhydrous calcium chloride.

9. Effect of various additives on virus infectivity :

To work out whether the stability and infectivity of virus will get increased. Several additives (Sodium sulfite, DIECA, EDTA, Sodium thioglycollate and mercapto-ethanol will be used. In case, the infectivity get enhanced, the most suitable additive will be selected and routinely added to the medium for virus exraction.

10. Purification : After selecting a suitable buffer a propagation host(s), an assay host(s) and biophisical properties, attempts will be made to purify the virus under consideration.

10.1 Clarification of Sap :

The infected leaves of the propagation host will macerated by usual and suitable method and the macerate

will be passed through a double layered cheese cloth. The sap thus obtained will be given a low speed centrifugation at 5000 g for 10 min. The supernatent (sap) will be subjected to various clarification procedures.

(a) **Celite and Charcoal** : - Celite and activated charcoal will be mixed with sap at the rate of 5g/100 ml., either separately or, in combination. when both are to be used 5g of activated charcoal will be mixed with 100 ml of sap and after ½ minute stirring 5g of celite will be added. Shaking will be removed by the following methods.

i. Centrifugation at 2,000 rpm for 5 minutes;

ii. Filteration through Buchner funnel supported by a 2-3 mm thick celite pad and filter paper (Whatman No. 1) or,

iii. Filteration through a filter paper (Whatman No.1) only in a Buchner funnel.

(b) **Organic Solvent**: Organic solvent (butanol, ethyl alcohal, chloroform, carbon tetrachloride and di-ethylether) either separately or in combination such as (chloroform-butanol) will be used in two ways for the removal of the extraneous plant material from the infected tissue.

i. by macerating the infected tissue by using a mixture of suitable buffer and organic solvents, or

ii. by adding requisite amount of solvent in crude sap obtained after macerating the infected tissue in buffer and filtering through two layers of cheese cloth.

The mxiture will be incubated for 20 min. and then centrifused at 5000g for 15 mm. The aqueous layer will be separated. The effect of solvent on the virus infectivity will be tested by assaying the aqueous layer for active virus content on a local lesion host.

(c) **Calcium phosphate gel** : The gel will be prepared by mixing 0.1M sodium dibasic hydrogen phosphate ($Na_2HPO_4 \cdot 2H_20$) and 0.1M calcium chloride (gel) in equal volume. The mixture after continuous stirring for 15 min. will be allowed to settle. Then the supernatent will be decanted. To the remaining precipitate double distilled water will be added and the resuspended precipitate will again be allowed to settle down. In this way precipitate (gel) will be washed 15-20 times to assure formation of antibodies as well as titre of the antiserum, antigen (virus preparation) will be injected intravenously or intramuscularly or in both ways.

The antigens will be administered intravenously through the marginal ear vein of the rabbit using a clinical syringe with a fine needle. Five to seven weekly injections of virus preparation of 2 ml each will be administered intravenously through the marginal vein of the ear. For intramuscular injections, antigens will be emulsified with an equal volume of Freund's incomplete adjuvant. Two injections of the virus adjuvant mixture of 3 ml each at an interval of 2 weeks will be administered intramuscularly in thigh of the same rabbit which has been given intravenous injections of antigens. Test bleedings will be made several times from the ear of the rabbit at different intervals after the administration of last intramuscular injections to check the antibody titre in serum. After the titre has reached its maximum, the immunized rabbits will be finally bled by giving a sharp incision on the marginal vein of the ear, which has not been used for injecting the antigen. About 10-15 ml of the blood will be collected and allowed to clot at room temperature ($20\pm5°C$) for 2 hrs. and kept the removal of chloride ions (Cl^-). Ultimately it will be equilibrated with phosphate buffer (0.1M pH 7.0). Such freshly prepared gel will be mixed with sap obtained after low speed (5000g for 10 min.) Centrifugation of the crude sap, stirred vigorously and centrifugation for 5 min. at 5000 g. The clear supernatent will be assayed for virus activity on local lesion host.

(c) **Silver nitrate** : Different volumes of 1 per cent silver nitrate solution will be added drop by drop to the standard inoculum (1/5) and stirred simultanously. The mixture will be left at room temprature for 30 min and thereafter, centrifuged at 5000 g for 15 min. The supernatent thus obtained will be bioassayed on local lesion host for virus infectivity.

Out of the clarification methods described above, one will be standerdized and used as clarification methods in the purification of the virus being studied.

10.2 Concentration of Virus :

The sap obtained after clarification treatment as described above will be used for concentration of virus by any of the following methods.

(a) **Differential Centrifugation** : The ultracentrifugation will be worked out in model L3-50 Backman preparative ultracentrifuge using rotor type-50. Normally, high speed centrifugation will be done at 97,000g unless otherwise stated. The pellet, thus obtained will be dissolved in a suitable buffer. Low speed centrifugation will be

performed at 10,000g in a Remi T-24 centrifuge or anyother same type of centrifuge. The number of cycles and the time of centrifugation at different rpm will be carried out keeping in view the stability of the virus and its sedimentation. Activity of different samples in supernatent and the pellet will be assayed on local lesion host.

(b) **Precipitation** :

(i) **Poly ethylene glycol (PEG)** : Poly ethylene glycol 6,000 will be used for precipitating the virus in clarified sap. Precipitation of the virus will be tried with 2, 4, 6, 8, 10 and 12 per cent PEG separately. In every case, the variation in salt (Nacl) concentration and its impact on precipation of the virus will be standerdized. After the addition of requisite quantity of PEG and Nacl to the clarified sap, the mixture will be stirred on a magnetic stirrer till both (PEG and Nacl) are dissolved completely and kept in a refrigerator at 4° to 8°C for 6 hrs to allow complete precipitation.

(ii) **Ammonium Sulphate** : Different quantities (10-40%) of ammonium sulfate $(NH_4)_2SO_4$ (W/V)

will be added to clarified sap (1/1). The mixture will be stirred at 8±2° in an ice bucket till the $(NH_4)_2SO_4$ crystals are dissolved completely. The mixture will then be incubated at 4±1°C for 2 hrs. and centrifuged at 5,000 g for 15 min to collect the precipitate.

The pellet thus obtained by PEG and $(NH_4)_2SO_4$ precipitation will be dissolved separately in a suitable buffer and recentrifuged at 5,000g for 5 min. Supernatent thus obtained will be assayed on local lesion host.

11. **Further Purification by density gradient Centrifugation** :

Concentrated virus samples obtained by the methods described above will be subjected to further purification using density gradient centrifugation (Brakke, 1951, 1960).

Linear sucrose gradient columns will be prepared by layering 7, 7, 7, and 4 ml of 0.1 M phosphate buffer pH 7.0 having 400, 300, 200 and 100 mg sucrose per ml, respectively, in a 1x3" tube. The sucrose solutions of

different concentrations will be layered using a pipette with a broad orifice.

The heaviest solution will be layered Ist and the solutions of decreasing concentration will be layered on the top of each other. The column will be used after standing for 24 hrs. in a refrigerator usually 2 ml of the virus preparation will be floated on the top of the column and the column will be centrifuged immediately after floating the virus preparation to avoid droplet sedimentation. The column will be centrifuged in Sw-25.1 rotor in L3-50 preparative ultracentrifuge. The acceteration upto a few hundred rpm will be made gradually. The tubes will be centrifuged for 2½-4 hours. After centrifugation the tubes will be examined in a dark room by projecting a beam of light down the tube from the top. The virus zone scattering the light will be removed from the tube by 20 guaze 10 cm long needle bent twice at right angles and attached to a hypodermic syringe.

12. UV-Spectrophotometry :

The virus preparation will be examined in Backman DU-2 model ultraviolet absorption spectrophotometer to evaluate the different methods of purification and to ascertain the purity virus isolated.

Ultraviolet radiations are absorbed in a characteristic manner by the virus (nucleo protein) containing solutions. Absorbance of samples will be studied in UV-range (230-320 nm) and graphs will be plotted. Values of A-max/min, A-280/260 and A-260/280 will be calculated to know the approximate percentage of nucleic acid.

13. Electron microscopy :

Shape and size of virus particles will be studied in transmission electron microscope.

13.1 Leaf dip method :

Method described by Brandes (1964) will be followed for leaf dip preparations. One drop each of 2% potassium phosphotungstic acid (PTA) and uranyl acetate will be placed separately on several formvar coated copper grids having carbon backing. The freshly cut ends of infected leaves will be dipped in the drop of 2-4 seconds. Such grids will be allowed to dry for sometime and thereafter examined under electron microscope at various magnifications.

13.2 Procedure with purified virus preparation :

A small drop of purified virus preparation will be placed on formvar coated copper grids having carbon

backing then a small drop of suitable strain (either PTA or uranyl acetate) will be added to the virus suspension. The excess fluid will be absorbed with a small piece of filter paper leaving a very thin film of fluid on the grids, which will be dried at room temperature. Such grids will be examined under TEM.

14. Serology :

Specific antigen and antibody reaction is one of the useful techniques either for assigning the viurs to a particular group or to differentiate it at the strain level. Antisera to the virus under consideration will be prepared for the identification of the virus as well as for testing the latent infection in certain hosts. Besides, it would also useful for ascertaining the seed transmissible nature of the virus through routine serological methods or by enzyme - linked immunosorbent assay (ELISA).

14.1 Raising of antisera :

Young healthy rabbits, approaximately 3 lbs. in weight will be used for production of antisera. The purified or partially purified virus preparation will be used as antigen.

To work out the effect of injection on the overnight in a refrigerator. Serum containing antibodies (antiserum) will thereafter be separated and centrifuged at 1,000 rpm for 5 min. to remove fibron, blood cells etc. The straw yellow coloured antiserum will be collected and stored for serological studies.

To identify the virus under investigation upto group or strain level, the Ouchterlony's double diffusion test (Ouchterlony, 1962) will be performed.

14.2 Ouchterlony double diffusion test :

One per cent agar will be prepared in 0.85% saline containing 0.1-0.2% sodium azide. Suitable amount of agar will be poured into sterilized petridishes so as to get a 2-3 mm thick agar bed. Using a cork borer of 5mm diameter well will be made into the agar and the cut portions of agar will be removed by aspiration. The distance between two wells will be kept 5 mm apart. The central well will be filled with antiserum. The remaining wells will be loaded with various dilutions of antigens made in physiological saline (0.85% Nacl solution, and the clarified sap from healthy plant. Such treated petridishes will be incubated at room temperature and absorved for formation of precipitation lines.

14.3 Precipitin test in tubes:

Equal proportions of antiserum and antigen after making two fold dilutions in 0.85% saline will be mixed togather in serological tubes (6x1cm) and incubated at 37°C in a water bath. The formation of precipitate and its intensity will be observed using a magnifying lens.

15. Isolation of nucleic acid :

Phenol detergent method will be used to isolate the nucleic acid of viruses. To a 2.5 ml of purified virus preparation will be added 0.05 ml of 6% sodium dodecyl sulphate and 2.6 ml of water saturated phenol. The phenol used will be redistilled and stored at 10°C after adding distilled water. The mixture will be stirred in a glass tube on a magnetic stirrer for a 10 minutes and then centrifuged for 5 minutes at 3,000 rpm in a clinical centrifuge. The mixture will be separates into two layers, the upper aqueous layer and the lower phenol layer containing sodium dodecyl sulphate. The top aqueous layer willbe drawn with a pipette. To the lower phase 2.5 ml of 0.1M phosphate buffer pH 7.0 will be added and stirred for 10 minutes and then centrifuged for 5 min. at 3,000 rpm. The aqueous phase will be drawn off and pooled togather with the aqueous phase obtained at previous step and stirred for 10 min. with an equal volume of phenol followed by centrifugation. The aqueous phase will be

extracted once more with half the volume of phenol. Traces of phenol will be removed from the aqueous phase by extraction with ether. The nucleic acid will be precipitated by the addition of 2 ml of ice-cold ethanol to the solutions. The precipitate will be pelleted out by centrifugation for 15 minutes at 7,500 rpm. The pellet will be suspended in 0.1M phosphate buffer pH 7.0 and centrifuged for 15 min. at 10,000 rpm to remove any insoluble material present in the precipitation, and the supernatant thus obtained will be tested for infectivity and type (RNA or DNA) of the nucleic acid.

15.1 Infectivity of viral nucleic acid :

The infectivity of viral nucleic acid will be assessed by inoculating the nucleic acid preparation on local lesion host. Several dilutions of nucleic acid preparation will be made and inoculated on the local lesion host and the number of local lesions developed will be compared with the corresponding dilutions of the virus preparation.

15.2 Type of nucleic acid :

It is well known that RNA and DNA differ in their chemical composition with respect to the base and sugar

involved in their composition. RNAs are knows to contain ribose sugar and uracil base (other three bases being adenine, cytosine & guanine) while DNAs contain thymine (Other three bases are same as in case of RNA) and deoxyribose sugar.

Thus, test will be performed to study the type of sugar. Diphenylamine test for deoxyribose or orcinol test ribose sugar will be used for ascertaining the type of nucleic acid in virus under investigation.

16. Studies on proteins of the virions :

Attempt will be made, while using standard methods to determine the approximate molecular weight of proteins associated with the virions.

****

CHAPTER - 4

REFERENCES

R E F E R E N C E

Anderson C.W. (1954). Cassia tora, a leguminous host of tobacco etch virus. Plant Dis. Report. 38,(II), pp 736-738.

Bald J.G. and Samuel G. (1931). Investigations on 'Spotted wilts' of tomatoes. Austratia Council Sci.& Indus. Res. Bull. 54, 24 pp.

Beale Helen P., (1934). The serum reactions as an aid in the study of filtrable viruses of plants. Contrib. Boyce Thompson Inst., vi, 3, pp. 407-435.

Benson, A.P.; Raymer, W.S.; Smith, W.; Jones, E. and Munro, J. (1965). Potato diseases and their control. Coll. Agric. New Brunswick N.J.

Chamberlain, E.E. (1937). Tobacco mosaic. Its appearance, cause and control. N.Z.J. Agric., IV, 3 pp. 163-174.

Costa, A.S. (1944). Quantitative studies with carborundum and its use in local lesion tests. Phytopathology, XXXIV, 3, pp. 288-300.

Deighton, F.C. (1929). Report on the mycological section. Ann. Report. Lands & Forests. Dept. Sierra Leone for the year 1929, pp. 14-19.

Doolittle, S.P. and Walker, M.N. (1925). Further studies on the overwintering and dissemination of cucurbit mosaic. Journ. Agric. Res. 31, 1, pp. 1-58, 6 pl. Madeson wiscosine, U.S.A.

Fribourg, C.E. (1979). Host plant reactions some properties and Serology of Peru tomato virus. Phytopathology 69(5) 441-445. Universidad Nacional Agraria, Lima Peru.

Gardner M.W. (1927). Indian Plant diseases, 1925. Proc. Indian Acad. Sci., XXXVI, pp. 231-247.

Graca, J.V. DA; Tranch, T.N.; martin, M.M. (1985). Tomato Spotted wilt virus in Commercial Cape-gooseberry (Physalis peruviana) in Transkei. Plant Pathology. 34(3) 451-453. Dep. Microbiol. Pl. path., Univ. Natal, Pietermaritzburg 3200, South Africa.

Holmes, F.O. (1942). Quantitative measurement of a Strain of tobacco etch virus. Phytopathology, XXXII, 12, pp. 1058-1067, 1 graph.

Horvath , J. (1970). Reaction of Physalis species to Plant viruses I. The cape - gooseberry as a symptomless carrier of potato virus x and y. Acta. Phytopath. Acad. Sci. Hung. 5(1), 65-72. Res. Inst. Pl. Prot. Budapest, Hungary.

Horvath, J. (1974). Reaction of Physalis species to plant viruses, II. Additional data on the virus susceptibility of Physalis spp. and new experimental results. Physalis peruviana L. var. macrocarpa and P. peruviana L. Acta Phytopathologica Academiae Scientiarum Hungaricae, 9(½) 1-9. Res. Inst. Plant Prot. Budapest, Hungary.

Horvath, J. (1974). Reaction of Physalis species to plant viruses. III. P. floridana Rydb. and P. peruviana L. as immune and screening Plants to several viruses. Acta phytopathologica Academiae Scientiarum Hungaricae, 9(½) 11-15. Res. Inst. Plant Prot., Budapest, Hungary.

Johnson J.(1926). Mosaic diseases on differential hosts. Phytopath., 16, 2, pp. 141-149, 5 Pl.

McClean A.P.D. (1931). Bunchy top disease of the tomato. S.Africa Dept. of Agric., Sci. Bull. 100, 25 pp, 8 Pl.

McClean A.P.D. (1931). Bunchy top disease of tomato. Farming in South Africa, VI, 67, pp. 275-276, 280.

McClean A.P.D. (1935). The Bunchy top disease of tomato. Host range of the Bunchy top of virus. Fmg. S. Afr., X., 112 pp. 302-303.

Mc Clean A.P.D. (1940). some leaf curl diseases in South Africa (1) Leaf curl disease of tobacco (ii) A new 'petunia' strain of leaf curl and a note on the occurence of a leaf curl disease of Holly-hock. Scien. Bull. Dep. Agric., S. Afr. 225, 75 pp., 14 Pl., 1 fig.

Mc Keen C.D. (1954). Inhibition of virus infections of certain plants by extract from capsicum frutescense L. Science, 120, 3110, p . 229.

Mc Keen C.D. (1956). The inhibitory activity of extract of Capsicum frutescense on plant virus infections. Canad. J. Bot., 34, 6, pp. 891-903.

Muriel J. O'Brien & W.B. Raymer (1964). Symptomless hosts of the potato spindle tuber virus. U.S.D.A. Beltsville, Md. 884.

Nariani T.K. & Pathanian P.S. (1954). Physalis peruviana L. a new host of tobacco leaf curl virus. Indian Phytopath., 6,2, pp 143-145.

Nariani,T.K. & Sharma, B.C. (1971). A mosaic disease of cape- gooseberry (Physalis peruviana) caused by cucumis virus, I. Indian Phytopath. 24 (2): 383-384, IARI, New Delhi.

Natti, J.J.; Kirkpatrick, H.C. and Ross, A.F. (1953). Host range of potato leaf roll virus. Amer. potato J., 30, 3, pp 55-64.

Sakimura, K. (1953). Potato virus y in Hawaii. Phytopathology, 43, 4, p. 217.

Schemelzer, K. (1955). A contribution to the knowledge of the host plant range of the tobacco stem mottle virus. Naturwissenschaften, 42, 20, p. 564.

Singh, R. (1968). A mosaic disease of cape- gooseberry (Physalis peruviana L.). Sci. Cult., 34(10): 424. Univ. Gorakhpur, Utter Pradesh.

Singh, S.J.; Sastry, K.S.M.; Sastry, K.S. (1975). Investigations on a mosaic disease of cape-gooseberry. Current Science, 44(3) 95-96. Indian Inst. Hort. Res. Bengalore, India.

Singh, R.P. (1978) Detecting virus A in potatoes. Canada Agriculture, 23(3) 16-18. CDA, Res. Sta. Fredericton, NB., Canada.

Walker, M.N. (1925). the relation of certain species of Physalis to the overwintering of the mosaic disease of cucumber. Phytopath., 15,12, pp. 733-744, 2 Pl. U.S.A.

****